EXPERIMENTAL BIOLOGY

A STUDY OF THE INFLUENCE OF TISSUE FLUID ON THE NUDIBRANCH MOLLUSC (Aeolidia papillosa) ON THE COURSE OF DEVELOPMENT OF THE MOLLUSC

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In recent times the concepts and methods of immunology have acquired increasingly wide application in various branches of biology, particularly in embryology. N. N. Zhukov-Verezhnikov [5] has advanced the hypothesis of primary immunological reactivity, according to which newly-formed protein plays the role of antibody with respect to the proteins which have served for its synthesis. A particular result of this interaction between the "new" and "old" proteins in the developing organism is the formation of normal antibodies. This viewpoint has received confirmation in recent works [1, 3, 4].

According to N. N. Zhukov-Verezhnikov, one piece of evidence for the presence of primary immunologic reactivity in animals and in man is the discovery of normal antibodies in any animals which have not been previously immunized by the corresponding antigen.

We have set out to determine the role of normal anitbodies in the formative processes. It seemed important to determine firstly whether tissue fluids of the nudibranch mollusc (Aeolidia papillosa) contains normal precipitins against tissues of embryos and larvae of Aeolidia, and secondly how such tissue fluids act during the course of individual development of the mollusc.

EXPERIMENTAL METHOD AND RESULTS

Adult mollusc were collected from the littoral zone at low tide and placed in an aquarium containing sea water. Under these conditions eggs were laid. The subsequent development of the embryos proceeded quite normally.

From the body cavity of the mollusc which had laid eggs we collected tissue fluid which was then kept for 18 h at 4°*. The fluid was then centrifuged for 20 min, and used for the experiment.

From embryos at the stage of cleavage and from early veliger larvae extracts were prepared consisting of four volume of filtered sea water to one volume of a suspension of embryos or larvae. After the homogenates obtained in this way had been extracted for 18 h in the cold they were centrifuged and the supernatant fluid was used in the experiment.

The tissue fluid of an adult mussel and extracts from embryos and larvae were used in the ring precipitation test. In the control experiments filtered sea water was added as a layer on top of the tissue fluid or extracts.

From the results shown in Table 1 it can be seen that tissue fluid diluted 1:2 reacted with 1:50, 1:100, and 1:200 extracts of embryos at the cleavage stage. Similar results were obtained in experiments with tissue fluid, and with an extract of early veligers. In control experiments the precipitation ring was never observed. The results were

*As has been shown previously [3] the preservation of tissue fluid for 12-18 h in the cold is required in order to destroy cells contained in this fluid and for the liberation of the normal antibodies which they contain.

TABLE 1. Results of the Ring Precipitation Test Between the Tissue Fluid and Extracts from the Tissues of Embryos or Larvae of the Same Species of Mollusc

Reagent diluted 1:2	Source of tissue extract	Dilution of extract	Results
Tissue fluid of adult mollusc	Embryos at the cleavage stage	1:50	++
		1:100	++
		1:200	+
	Larvae—early veligers	1:50	++
		1:100	++
		1:200	+
Sea water (control)	Embryos at the stage of cleavage	1:50	0
		1:100	0
		1:200	0
	Larvae—early veligers	1:50	0
		1:100	0
		1:200	0
	Adult molluscs (tissue fluid)	1:2	±

TABLE 2. Influence of Tissue Fluid of an Adult Mollusc on the Course of Development of Embryos of the Same Species

	Stage of development	Dilution of tissue fluid	Results
Experiment	Division (4, 16, 32 blastomeres)	1:15	Inhibition of development
		1:20	Same
		1:30	"
	Blastula	1:15	"
		1:30	"
	Veligers (early, intermediate, or before hatching)	1:15	"
		1:20	"
		1:30	"
Control	Division (4, 16, 32 blastomeres)		Normal development
	Blastula		Same
	Veligers (early, intermediate, or before hatching)	Sea water	"

evaluated for 30 min. The experiment was repeated six times with each extract indicated in Table 1, and with tissue fliud. In all cases we obtained similar results.

Thus in the tissue fluid of an adult mussel substances are present which have the power to react in vitro with tissue extracts of embryos or larvae of this species of mollusc. We are inclined to regard such substances as normal antibodies.

In a study of the action of tissue fluid on the course of individual development of this mussel we added tissue fluid taken from an adult mussel to sea water to obtain the following dilutions: in the first dish—1:15, in the second—1:20, in the third—1:30, and in the fourth dish which was the control we added sea water only. Next into each dish we placed a portion about 20 mm of an egg batch which contained about 400 egg capsules with embryos at a particular stage of development. The medium in the dishes was changed daily.

To evaluate the results we made observations on the course of development of the embryonic nudibranches under the microscope. Observations were made daily at 6-h intervals for 2-9 days.

TABLE 3. Influence of Immune Serum Against Tissues of Molluscs or of Normal Rabbit Serum on the Course of Development of the Embryo Mollusc

Stage of development	Substance added to the sea water	Dilution	Results
Early veligers	Immune serum against mollusc tissue	1:100 1:1000 1:10,000	Death of embryos Ditto Normal development
Early and late veligers	Normal rabbit serum	1:100 1:1000 1:10,000	Normal development Ditto "
Early and late veligers			Normal development

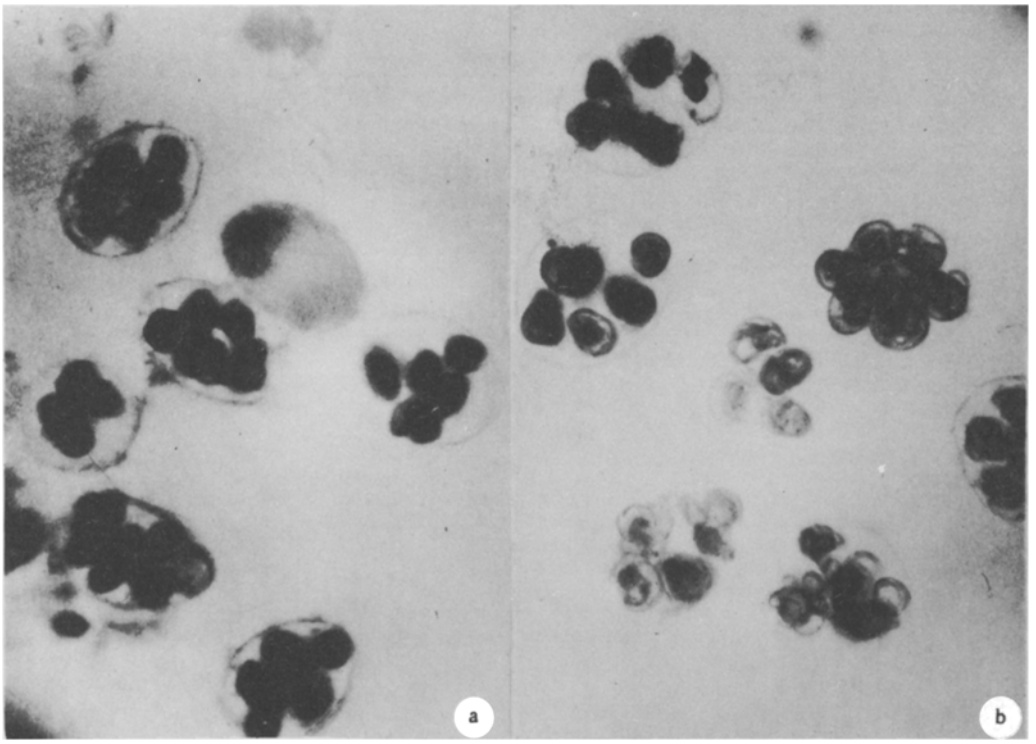

Fig. 1. Influence of tissue fluid on veligers. a) Experiment: veligers smaller than in the control have an abnormal form and structure; shells but little transparent; death and disintegration of veligers; b) control; average veligers. Magnification 70 ×.

As can be seen from Table 2 and Fig. 1 in every case the tissue fluid of the adult mussel exerted an inhibitory influence on the course of subsequent development of the embryos taken at the cleavage stage (4, 16, or 32 blastomeres), or at the blastula stage, or from veligers at various stages of development.

In the experiment in which we used embryos at the 4-blastomere stage, we noted a marked deviation from normal development at $3\frac{1}{2}$ days. However in experiments in which we used embryos at later stages of 16, or 32 blasomeres, or blastulae, noticeable deviations were found after three and after $2\frac{1}{2}$ days. In all these cases the development proceeded to the stage of formation of the veliger. The onset of movement of the embryos was recorded much later in the dishes containing tissue fluid than it was in the controls. The nature of this movement also indicated a marked arrest of embryonic development. Whereas in the controls we noticed an active rotational movement of the embryos, in the dishes with tissue fluid we found a scarcely noticeable twitching. Large doses of tissue fluid had a

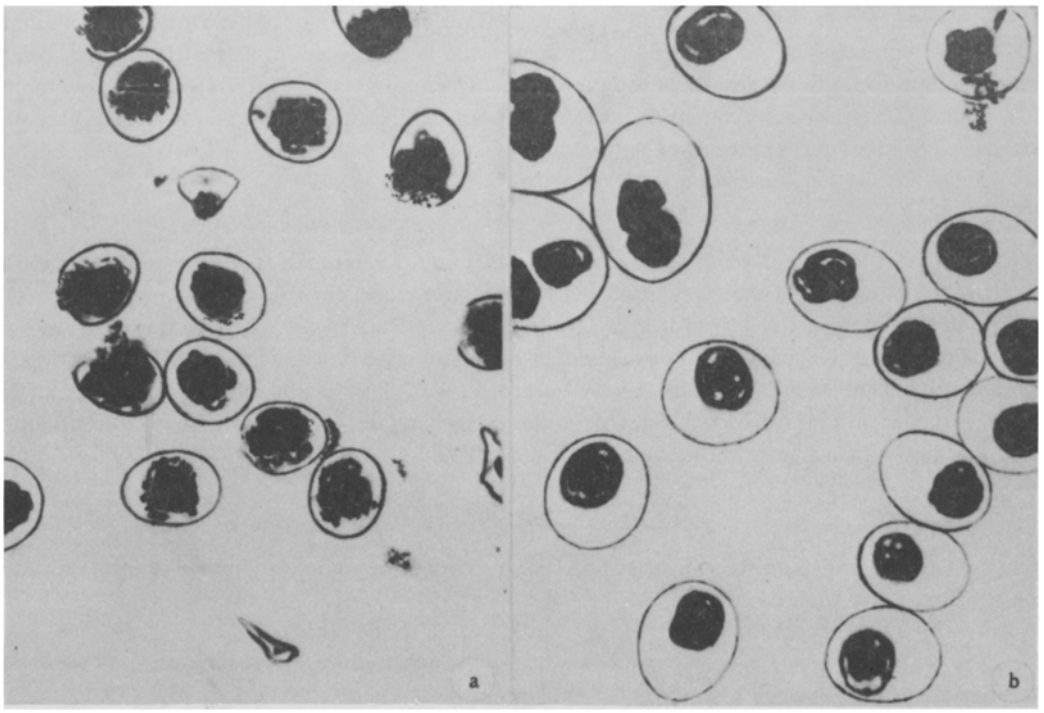

Fig. 2. Influence of immune antibodies on veligers. a) Experiment: disintegration of veligers observed; b) control: early veligers. Magnification 70 ×.

more marked inhibitory action. In the case of the action of tissue fluid on veligers, the latter did not hatch from the egg capsule. Furthermore the veligers were retarded in growth, being much smaller than the controls. Most of them acquired an abnormal spherical shape. In certain cases they died, and disintegrated completely.

For comparison of the results of the influence of tissue fluid with those obtained by means of immune antibodies we carried out corresponding experiments: into a series of dishes containing sea water we added rabbit serum immune to mollusc tissue, in dilutions 1:100, 1:1,000, and 1:10,000. In this case we used two controls: in one control dish, besides sea water we added nothing, and in the other we added normal rabbit serum in the same dilutions as the immune serum. Then we also placed sections about 20 mm long of the batch of eggs containing larvae at the early veliger stage. Observation on the course of development of the larvae was continued for six days.

As can be seen from Table 3 and Fig. 2, normal rabbit serum exerts no influence on the development of veligers whereas immune serum in dilutions of 1:100 and 1:1,000 causes death and destruction within a few days.

Thus the influence of tissue fluid on the course of development of mollusc embryos was similar to the influence of immune sera. This gave us reason to suppose that the active factor in the tissue fluid is related to normal antibodies, because the latter, as was pointed out above, were found in tissue fluid.

The results of the present investigation carried out on the nudibranch mollusc Aeolidia papillosa confirm existing reports on the presence in animal tissue fluids of substances which react as antibodies with extract from tissues of embryos at earlier developmental stages [1, 6]. In our experiments we also found that the tissue fluid of Aeolidia, containing these normal precipitins against embryonic tissues exerts an inhibitory influence on the course of development of the embryo. Here the action of the tissue fluid resembled that of immune sera, as was shown both by our own and by published results [2, 7-10].

In view of these facts we hold that normal precipitins contained in the tissue fluid of adult molluscs exerts an important influence on the course of development of the embryo.

Indeed the development of the embryo maybe conceived as a chain reaction consisting in the gradual formation of a strictly determined series of protein structures. According to the theory of primary immunological reactivity, at a particular moment in the synthesis, newly-formed protein blocks specific protein (nucleoprotein) which serves as the basis for its synthesis.

In our experiments, in our study of the action of tissue fluid on the course of development of the mollusc we introduced these "new" proteins before the time when they would normally be formed. Under these conditions they evidently blocked prematurely the synthesis of the corresponding protein, and so interfered with development. Possibly during the course of normal embryogenesis these substances, being antibodies in nature, take part in mechanisms which regulate the developmental processes of the embryo.

SUMMARY

A study was made of the effect produced by tissue fluid from a nudibranch mollusc (Aeolidia papillosa) on the course of individual development of the same species. Normal antibodies capable of reacting with the the antigens of the tissue of Aeolidia embryos and larvae were found in the tissue fluid of the adult molluscs. Tissue fluid containing these antibodies inhibited subsequent development of the embryos taken at the stage of cleavage, blastula, and larvae. The results of the tissue fluid effect were compared with those of the action of immune antibodies against the mollusc tissue on the course of the development of the larvae. An attempt has been made to explain the data in terms of the theory of primary immunological reactivity.

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